

**Faster acquisition of symbiotic partner by common mycorrhizal networks in early plant life stage**

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## Abstract

Arbuscular mycorrhizal (AM) fungi usually improve plant performance yet our knowledge about their effects on seed germination and early plant establishment is very limited. We performed a factorial greenhouse experiment where the seeds from four low Arctic co-occurring mycorrhizal herbs (*Antennaria dioica*, *Campanula rotundifolia*, *Sibbaldia procumbens*, and *Solidago virgaurea*) were germinated alone or in the vicinity of an adult *Sibbaldia* plant with or without AM fungi; given either as spores or being present in a common mycorrhizal network (CMN). Three different AM fungal species were examined to assess species-specific differences in symbiont acquisition rate. Out of the four plant species investigated, the presence of AM fungi affected seed germination only in *Campanula* and this effect was dependent on whether the AM fungi were present in the soil as spores or as a CMN. Overall, after germination, developing seedlings showed AM fungal colonization in their roots as soon as 2 days after cotyledon emergence. Our results show that CMN may provide germinating seedlings faster acquisition of the AM fungal partner in comparison to acquisition from spores. Furthermore, there were AM species-specific differences in the symbiont acquisition rate highlighting the importance of species identity in AM interactions. These findings suggest that while AM fungi may not play a fundamental role during seed germination, plant community composition may be affected by the species-specific AM fungal effects on seedling establishment and CMN acquisition.

**Key words:** *Antennaria dioica*; arbuscular mycorrhizal fungi; *Campanula rotundifolia*; common mycorrhizal network; seed germination; seedling establishment; *Sibbaldia procumbens*; *Solidago virgaurea*.

## **Introduction**

Our knowledge of the role of mycorrhizal symbiosis on the early part of plant life cycle is very limited in contrast with the well-established effects of mycorrhizal symbioses on plant growth and reproduction later in plant life (Smith and Read 2008, Koide 2000). In most plant communities, seedling recruitment is limited by several abiotic and biotic factors including competition from established vegetation (Moles and Westoby 2004) and therefore seed germination and seedling establishment will partly determine community composition. In natural communities, developing seedlings may immediately contact with arbuscular mycorrhizal (AM) fungi present in the soil. AM fungi are ubiquitous symbiotic organisms growing in association with the roots of 73% of angiosperms (Brundrett 2009). AM fungi may connect roots of several coexisting plant individuals forming the so-called common mycorrhizal network (CMN, Newman 1988). Even though AM fungi are usually shown to be beneficial for plants, a review of the limited available literature suggests that their effects on early plant stages are variable (Van Der Heijden and Horton 2009, Merrild et al. 2013) and several studies have shown that AM fungi have neutral or even negative effects on seedling recruitment. The negative effects of AM on developing seedlings have been ascribed to the carbon costs associated with sustaining the fungi (see (Johnson et al. 1997) and references there). Especially for small seeded species where the amount of available resources in the seed may be limited or when P availability does not constrain plant growth, AM fungi may represent more a cost rather than a benefit for the developing seedling (Koide 1985, Ronsheim 2012).

In contrast to the well-established effects of the presence of host plants on AM fungal spore germination (e.g. Parniske 2008), the effects of AM fungi on host seed germination are relatively understudied. Seeds acquire information about the environmental conditions such as resource levels (Aphalo and Ballare 1995). Because AM fungi usually improve seedling

survival and especially so under harsh environmental conditions, it seems plausible to assume that the presence of AM fungi (both in the form of spores or CMN) should also increase host germination and that seeds could be able to detect the presence of AM fungi. To our knowledge, the recognition of AM fungal presence by seeds has not been verified previously.

Seedling mortality is high in nature and most seedlings in temperate ecosystems perish due to post-dispersal seed predation and low water availability in top soil (Moles and Westoby 2006). Racing against time after rainfall, seedlings may allocate resources to root systems in order to reach deeper and moister soil strata. AM is an alternative resource acquisition and allocation strategy that may benefit seedlings in their very critical first days (Van Der Heijden and Horton 2009). It is believed that developing seedlings get immediately tapped into the existing CMN, therefore gaining access to nutrients and water and possibly even carbon derived from other plants connected to the CMN, thus increasing their growth and chances for establishment (Van Der Heijden 2004). Connecting to CMN in contrast to stimulating AM fungal spore germination followed by extra- and intraradical mycelium construction has supposedly two benefits. Firstly, CMN may provide faster mycorrhiza formation in contrast to acquiring symbiosis from soil spore bank (see e.g. Püschel et al. 2007). Secondly, a large proportion of the construction costs of hyphal network can be avoided as the CMN is already there. However, neither of these has been proven experimentally.

It is well known that the identity of the AM fungi and plant species involved in the interaction as well as the biotic and abiotic factors both partners experience are important determinants for the outcome of the mycorrhizal relationship (Hoeksema et al. 2010). For example, not all AM fungal species deliver the same benefits in terms of nutrient transfer to the hosts and some AM fungal species may be more carbon demanding than others (Pearson and Jakobsen 1993, Lerat et al. 2003, Feddermann et al. 2010, Lendenmann et al. 2011). In

that regard, it has been shown that members of the Gigasporaceae have higher carbon requirements than *Glomus* species (Thomson et al. 1990). Differences may exist even within the same genus (see e.g. Graham et al. 1996). The different carbon demands are probably related to the intrinsic differences in the formation of the external mycelia and the intraradical development of each species (Chagnon et al. 2013). Thus, it is perhaps not surprising that the net outcome of the relationship for the plant with different AM fungal species is variable and may range from mutualism to parasitism (Johnson et al. 1997, Hoeksema et al. 2010), but see (Kiers et al. 2011). Theoretically, the same idea could apply to CMN formed by different AM fungi.

CMN may mediate plant resource competition (Merrild et al. 2013). Recently, the potential role of AM fungi on competition through allelopathy (i.e. the release of plant substances to the environment with negative effects on other plants) has been noted (Barto et al. 2011, Achatz et al. 2014). In these experiments, CMN enhanced the transport of substances across a range of distances, increasing the uptake of these compounds by a target plant (Barto et al. 2011). Several studies have demonstrated direct allelopathic effects mediated by AM fungi (see Sanon et al. 2009, Hale and Kalisz 2012) by facilitating transport processes and therefore extending the bioactive zone of allelochemicals in the soil, described as the Network Enhanced Bioactive zone model by the authors (Barto et al. 2012). However, no study has examined how AM fungi may affect allelopathic relations during seed germination.

Using a fully factorial greenhouse experiment, we germinated seeds from four plants in non-mycorrhizal conditions, with AM fungal spores or with an AM hyphal network connected to established adult plants. We specifically asked (i) how fast the roots from the developing seedlings become colonized by AM fungi germinating from spores vs. acquired from the CMN, (ii) whether the presence of AM fungal spores or a hyphal network affects

seed germination rate, and (iii) whether the presence of the established plant has any effect on seed germination.

## **Material and methods**

### Study organisms

Four common co-occurring low Arctic meadow plant species were chosen for the experiment: *Antennaria dioica* L. Gaertn. (Asteraceae), *Solidago virgaurea* L. (Asteraceae), *Campanula rotundifolia* L. (Campanulaceae), and *Sibbaldia procumbens* L. (Rosaceae). The seed material was collected in September 1999 from plants growing in a subarctic meadow at Kilpisjärvi, North Finland (698039 N, 208509 E) and stored dry at +4°C until germination took place.

The AM fungal isolates used were also of northern origin. *Glomus hoi* (BEG-104, referred as HOI hereafter) was isolated from the same location as the seed material; *Claroideoglomus claroideum* was originally isolated from an agricultural field in Muddusjärvi, Finland (69889 N, 27869 E, referred as CLA hereafter); and *Glomus boreale*-like isolate was collected near a glacier in Northern Norway (698349 N, 208109 E, referred as BOR hereafter).

### Experimental design

For the detailed experimental methods see Kytöviita et al. (2003). Briefly, to obtain established *Sibbaldia* plants, seeds were germinated in sterile sand and pre-grown under greenhouse conditions for 22 weeks. After that, seedlings were transferred into pots filled with 350 cm<sup>3</sup> of a 9:1 mixture of sterile sand and perlite with 5 g/L dolomite and 1.5 g/L bone meal (Honkajoki Oy, Honkajoki, Finland), containing 62 µg/g N, 180 µg/g P, 580 µg/g K, and 810 µg/g Ca. Each *Sibbaldia* plant was inoculated with 5 mL of substrate containing

abundant spores of one of the three AM isolates. Plants allocated to the non-mycorrhizal (NM) category were given 5 mL of identical substrate, but without spores. Three months after inoculation, the pots received a cold treatment of 12°C for five weeks and were brought back to the greenhouse. In spring 2000, when the *Sibbaldia* plants were 10 months old, they were transplanted into 12 x 13 x 6 cm plastic pots. The old potting mixture was carefully removed, plant roots washed and the plants planted into pots containing new potting mixture and grown for 8 weeks to allow hyphae to grow out of the roots and explore the substrate. The old potting mixture containing new spores of the three AM isolates was stored at room temperature for later use. Pots without established *Sibbaldia* plants were also prepared and treated identically to those containing plants. All pots were transferred at this point to Oulu University Botanical gardens greenhouse and grown under 18 h light: 6 h darkness photoperiod, and a temperature range between 15°C night time to 23°C day time.

Seeds from the four plant species were placed into nylon bags (mesh size = 0.50 µm) and stratified (4°– 8°C) for six weeks in a greenhouse and kept moist at 6°C before the experiment and the spores from the old potting mix were extracted by wet-sieving and decanting and collected onto a 54 µm sieve. The inoculum was diluted appropriately to obtain 100 spores/mL solution. The solution obtained from NM potting mixture was autoclaved at 120°C for 15 minutes to prevent any inadvertent contamination in the NM inoculum from spreading and stored for one week before being used. No Glomalean spores were observed in the NM control inoculum and none of the plants allocated to the NM treatments had any AM structures.

The experiment was initiated at the end of March 2000. At that point, 20 mL of the inoculum solution (containing about 2000 spores in the 3 mycorrhizal treatments) was evenly pipetted on the pots that contained no adult *Sibbaldia* plants. The packets with stratified seeds were each gently mixed in 40 mL water and were dispensed on pots. Each pot received only

one plant species consisting of either 75, 204, 584, and 718 seeds of *Sibbaldia*, *Solidago*, *Antennaria*, and *Campanula*, respectively. The number of seeds dispersed in each pot was calculated from a preliminary germination experiment aiming at obtaining about 30 seedlings per pot. All pots were covered with a layer of 2-3 mm of sterilized sand and were placed in plastic trays. Thus, the experiment was a factorial design containing pots with and without established *Sibbaldia* plants, one of the four fungal treatments (CLA, HOI, BOR, NM) and with seeds from one of the four plant species (*Antennaria*, *Campanula*, *Sibbaldia*, *Solidago*). In total, we had 478 experimental pots.

The pots were kept well watered by spraying deionized water on the pots about six times per day. Seedlings started emerging four days after sowing and they were monitored every two to three days. In the previous experiment, a cohort of 5–6 seedlings per pot were selected and harvested after reaching 37 – 54 days and all other emerging seedlings were recorded and were gently removed with forceps (Kytöviita et al. 2003). In the present experiment, we report the total germination success and AM fungal colonization in seedlings harvested 2 or 6 days after seedling emergence (referred as 2 and 6-days old seedlings hereafter) and not included in the previous report.

All seedlings were removed and kept in 80% ethanol until examination of AM root colonization with trypan blue. Briefly, entire seedlings were placed in KOH 10% for 15 hours, followed by 2 hours in an alkaline solution of 1.5% hydrogen peroxide to remove root pigments. After that, seedlings were acidified in HCl 1% for 2 hours and stained in 0.05% trypan blue for 2 hours at 80°C. After removing the excess staining in glycerol for 3 days, all seedlings were mounted on microscope slides and the presence of AM fungal structures was observed under 100x and 400x magnification. Because of the relatively small size of the root samples, AM root colonization was assessed and it is expressed as presence/absence of AM structure in the whole seedling. Due to the low germination of *Solidago*, few 2-days old



seedlings were harvested ( $n = 11$ ) and thus, data on AM fungal colonization were only analyzed on 6-days old seedlings.

### Statistical analyses

All statistical analyses were performed using R (R Core Team 2014). First we investigated whether the proportion of germinated seeds was affected by any of the explanatory variables (fungal treatment: NM, CLA, HOI, BOR; plant identity: *Antennaria*, *Campanula*, *Sibbaldia*, *Solidago*; presence of an established *Sibbaldia* plant: yes/no) using analysis of variance. A generalized linear model with a quasibinomial distribution was fitted to the data to correct for over-dispersion. All interactions between explanatory variables were included. After that, the response of each plant species was analyzed separately using two-way ANOVAs with fungal treatment and the presence of an established *Sibbaldia*. Models were assessed by visual inspection of the residuals. Differences between levels were investigated with Tukey's pairwise comparisons using the 'multcomp' package (Hothorn et al. 2008).

Differences in the proportion of seedlings colonized (yes/no) with AM fungal structures per pot were analyzed after fitting a generalized linear model to the data with binomial or quasibinomial (if data were over-dispersed) distributions as described above. Seedlings grown in non-mycorrhizal conditions were not included in the statistical analyses as they were not given mycorrhizal inoculum and were not mycorrhizal. All interactions between explanatory variables (fungal treatment: CLA, HOI, BOR; plant identity: *Antennaria*, *Campanula*, *Sibbaldia*, *Solidago*; presence of an established *Sibbaldia*: yes/no; and seedling age: 2, 6-days old) were included. After that, data on each plant species were analyzed separately. Due to the low number of *Solidago* seeds germinating and the 2-days old *Solidago* seedlings harvested ( $n = 11$ ), data on AM fungal colonization were only analyzed on 6-days old *Solidago* seedlings. Significant interactions were investigated with Tukey's pairwise

comparisons using the ‘multcomp’ package (Hothorn et al. 2008). Means  $\pm$  1 SE are presented throughout the paper.

## Results

### Seed germination

The four plant species used differed in the proportion of seeds that germinated (Table A1). *Sibbaldia* had the highest germination ( $52.9\% \pm 1.2$ ) compared to the other three plant species ( $16.2\% \pm 0.5$ ;  $14.3\% \pm 0.4$ ; and  $11.8\% \pm 0.4$  for *Campanula*, *Solidago* and *Antennaria* respectively; Fig. 1). The presence of a CMN affected seed germination in contrasting ways for each plant species as shown by the significant interaction between established *Sibbaldia* and plant species (Table A1). Furthermore, the effects of the CMN on seed germination varied among AM fungal species as shown by the significant interaction between these two factors (Table A1).

When analyzed separately for each plant species, neither the presence of an established *Sibbaldia* or the fungal treatment affected the proportion of germinated *Antennaria* seeds (Table 1, Fig. 1A). However, the presence of an established *Sibbaldia* decreased the overall proportion of *Solidago* seeds (Table 1, Fig. 1B) and also decreased seeds germination of conspecific *Sibbaldia* (Table 1, Fig. 1C), while no significant differences among fungal treatments or an interaction between these two factors were detected (Table 1).

The presence of an established *Sibbaldia* affected germination in *Campanula* seeds in a fungus-specific manner (Table 1). Pairwise comparison indicated that in NM condition, the established *Sibbaldia* reduced *Campanula* germination ( $P < 0.01$ ) and this effect was not mitigated by *G. boreale* ( $P = 0.01$ , Fig. 1D). However, when sown with *G. hoi* ( $P = 0.99$ ) or *C. claroideum* ( $P = 0.99$ ) the negative effects of the established plant were no longer observed and a similar proportion of seeds germinated regardless of the presence of an

established *Sibbaldia* plant (Fig. 1D). Compared to the germination of *Campanula* seeds germinated without an established *Sibbaldia* plant ( $22\% \pm 1$ ), seed germination was decreased by the presence of AM fungal spores of *C. claroideum* (to  $14\% \pm 1$ ) and *G. hoi* (to  $18\% \pm 1$ , Fig. 1D). Opposite to this, for seeds germinated with an established non-mycorrhizal *Sibbaldia* plant, seed germination was negatively affected by *G. boreale* (from  $15\% \pm 1$  to  $13\% \pm 1$ ,  $P = 0.03$ ) but it increased 2% with *G. hoi* ( $P < 0.01$ ), while *C. claroideum* had no significant effect ( $P = 0.06$ , Fig. 1D).

#### AM fungal root colonization

All four explanatory variables (presence of established *Sibbaldia*, the fungal and seedling species identity and seedling age) affected the proportion of seedlings colonized by AM fungi and significant interactions among these variables were also observed (Table A2). Overall, the proportion of seedlings colonized by AM fungi was larger in 6-days old than 2-days old seedlings (overall means  $23 \pm 2\%$  vs.  $6 \pm 1\%$  respectively). When 6-days old, *Sibbaldia* and *Solidago* had the largest proportions of seedlings colonized ( $29 \pm 3\%$  and  $32 \pm 4\%$  respectively) followed by *Antennaria* ( $16 \pm 3\%$ ) and *Campanula* ( $11 \pm 3\%$ ). Arbuscules were observed in 12 out of 240 2-days old seedlings (5% of all seedlings observed) and more than half of the seedlings (61%) had arbuscules when 6-days old. Because the proportion of root length colonized by arbuscules was very low in the 6-days old seedlings (average for *Antennaria*:  $3.9\% \pm 1.0$ ; *Campanula*:  $2.4\% \pm 0.8$ ; *Solidago*:  $9\% \pm 1.6$ ; *Sibbaldia*:  $7.3\% \pm 1.2$ ), data were not analyzed further.

When analyzed separately for each plant species, the proportion of *Antennaria* seedlings colonized by AM fungi was significantly affected by the seedling age, and the presence of an established *Sibbaldia* in the pot (Table 2). A larger proportion of seedlings became colonized as they grew older ( $1.5 \pm 0.6$  vs.  $15.8 \pm 2.9$  respectively, Fig. 2A) and the

presence of an established plant also increased the overall proportion of seedlings colonized (Fig. 2A). None of the seedlings grown with *G. boreale* spores became colonized at any age (Fig. 2A).

In the case of *Solidago*, only the 6-days old seedlings were analyzed and we found a significant interaction between the presence of an established *Sibbaldia* plant and the fungal treatment explaining the proportion of seedlings colonized by AM fungi (Table 2, Fig. 2B). The presence of a *Sibbaldia* plant increased the proportion of seedlings colonized when grown with *G. hoi* ( $P < 0.01$ , from 2% to 44%; Fig. 2B) and *G. boreale* ( $P < 0.01$ , from 0% to 42%; Fig. 2B). However, a similar proportion of seedlings had AM fungal structures in their roots regardless of the presence of established *Sibbaldia* when sown with *C. claroideum* ( $P = 0.62$ ).

In the case of *Sibbaldia* seedlings, the proportion of seedlings colonized was larger in 6-days old than in 2-days old seedlings ( $20\% \pm 4$  vs.  $11\% \pm 3$  respectively; Table 2, Fig. 2C) and there was a significant interaction between fungal treatment and the presence of an established *Sibbaldia* (Table 2, Fig. 2C). The presence of an established *Sibbaldia* increased the proportion of seedlings colonized only when sown with *G. boreale* (overall, from 0% to 19%;  $P < 0.01$ ; Fig. 2C, other  $P \geq 0.09$ ). Again, we did not observe any seedling colonized when grown with spores of *G. boreale*.

In *Campanula* seedlings, there was a significant three-way interaction between fungal treatment, seedling age and the presence of an established *Sibbaldia* explaining the proportion of seedlings colonized (Table 2; Fig. 2D). In 2-days old seedlings, the presence of *Sibbaldia* increased seedling colonization only when sown with *G. boreale* (from 0% to 4%,  $P = 0.06$ , all other  $P \geq 0.15$ ). In 6-days old *Campanula* seedlings, the presence of *Sibbaldia* increased seedling colonization when sown with *G. hoi* (from 0% to 20%,  $P = 0.04$ ) and *G. boreale* (from 0% to 20%,  $P < 0.01$ ; Fig. 2D).

## **Discussion**

In natural conditions seedlings become colonized by AM fungi soon after germination (e.g. Allen et al. 1989, Veenendaal et al. 1992), usually within days as seen in the present study. Theoretically, early AM formation enables seedlings to efficiently acquire water and nutrients from the surrounding soil, giving them an advantage over non-colonized seedlings (Smith and Read 2008). The speed of acquisition of AM may be critical under natural conditions where seedling mortality due to drought and biotic interactions is high. One of the putative benefits of CMN is that it allows faster acquisition of the mycorrhizal services (Van Der Heijden and Horton 2009, Simard et al. 2012) and that AM fungi are already supported by other plants, and therefore theoretically less likely to act parasitically on the seedlings. In this greenhouse study, 2-days old seedlings were already colonized by AM and arbuscules were observed in 5% of these seedlings. Overall, seedlings acquired symbiotic fungi faster from the network (measured as higher proportion of colonized seedlings), supporting the view that CMN may benefit developing seedlings. The spore density was high in the experiment and therefore the result is likely to reflect the time lag caused by the spore germination.

Kytöviita et al. (2003) showed that the performance of the solitary seedlings after one or two months was better than seedlings connected to a CMN. Taking both studies together, our results join others in showing that the effects of being tapped into a CMN strongly differ among plant and AM fungal species (see Selosse et al. 2006 and references there). In a recent meta-analysis, Moora and Zobel (2010) showed that competition out rules facilitative effects of CMN between plant life stages in experimental settings. It may be that experimental conditions designed to maximize network connectivity amplify the competitive aspects of CMN while under natural conditions seedlings are not that strongly connected to one strong competitor. For instance, when given a choice, seedlings may preferentially associate with

other AM species than adult plants thus reducing CMN connectivity. Usually developing seedlings get colonized by several AM fungal species through the growing season (e.g. Dumbrell et al. 2010). The relationship between plants and AM fungal partners within CMN can vary throughout the growing season in the field in terms of number of links between partners and shifts in associations (Bennett et al. 2013). In this context it is noteworthy that the *Glomus boreale*-like isolate only colonized *Solidago* seedlings through CMN. The solitary *Solidago* seedlings did not form mycorrhiza with this isolate in the presently reported 6-days or within previously reported 37-days old (Kytöviita et al. 2003). This shows that the plant or fungal ‘choice’ for partners may be affected by the CMN.

AM colonization rate (measured as the proportion of seedlings colonized by AM fungi per pot) was strongly related to the AM fungal species and the source of inoculum in our experiment. Whilst roots became quickly colonized by *C. claroideum* regardless of whether seedlings were grown only with spores or with an established *Sibbaldia* plant colonized by the fungus (and hence with external hyphae radiating from it), *G. hoi* and *G. boreale* appeared relatively more slow growing. Especially in the case of *G. boreale*, the proportion of seedlings colonized after 6 days was low compared to the other fungal species. In natural co-occurring AM fungal communities, these growth differences could translate into faster growing fungal species colonizing roots first. If the slower colonizing fungal species are competitively superior to faster ones, a succession of AM fungal communities through the growing season could be observed (Bennett et al. 2013).

Our results suggest a minor role of AM fungi during seed germination: out of the four plant species investigated, the presence of AM fungi affected seed germination only in *Campanula* and this effect was dependent on whether the AM fungi were present in the soil as spores or as a CMN. Moreover, our study indicates plant species-specific differences in AM acquisition in response to the established plant in the pot and the different AM fungal

species used. Positive effects on seed germination by AM fungi could be predicted, especially for dust-like seed species which may depend on the fungi to trigger germination and develop like myco-heterotrophic plants (Bidartondo 2005). However, the trend emerging from the very limited evidence (Table 3) indicates that the presence of AM fungal spores may have no or negative effects on seed germination and that this effect is not related to seed size in autotrophs (Fig. 3). In the present study, germination was not affected by AM fungal spores in *Antennaria*, *Solidago*, or *Sibbaldia*, whereas *Campanula* showed complex response to the fungal treatments. These results indicate that the presence of AM fungi generally does not affect seed germination, but when it does the effects are dependent on the plant – AM fungal species combination (Table 3). For example, Wu et al. (2014) evaluated seed germination of *Phragmites australis* using two different members of the Glomeraceae and showed similar results as the present study (Table 3): while *Rhizophagus irregularis* did not have any significant effect on seed germination, total seed germination was reduced by 12% when seeds were sown with *Funneliformis mosseae*.

As far as we know, only one study has reported positive effect of AM fungi on seed germination, even though the reasons behind such pattern were not discussed (Rueda-Puente et al. 2010). Similarly, the mechanisms behind the negative AM effect on seed germination in autotrophic plants remain unexplained. It has been shown that AM spore exudates are able to suppress seed germination, at least in some parasitic plants (Louarn et al. 2012). The communication mechanism between plants and AM fungi is complex and many processes are still unknown (see Nadal and Paszkowski 2013 for a recent review on the topic). To date, germinating spore exudates (GSE), some of which have been recently characterized as lipochito-oligosaccharides (e.g. Maillet et al. 2010), have been shown to stimulate root development and induce gene expression (see e.g. Mukherjee and Ané 2011). Whether GSE influences seed germination in non-parasitic plants remains to be explored. Altogether, the

lack of consistent results for the plant and AM fungal species used suggests that some species-specific mechanism may exist. There is the possibility that some unknown pathogen was introduced together with the AM fungal inoculum and affected *Campanula* seeds negatively. Several bacterial groups are found associated with AM fungal spores and whilst many are known to enhance seed germination and seedling growth (e.g. Hernández-Rodríguez et al. 2009) some species could theoretically act as antagonists for seed germination. Another plausible explanation for the plant-specific effects observed is linked to root structure. Plants with active, highly branched, rapidly growing, thin root systems are predicted to be the least responsive to AM fungi (Brundrett 1991). *Campanula rotundifolia* roots are thinner than those of *Solidago*, *Sibbaldia* and *Antennaria* (Coker 1966, Jonasson and Callaghan 1992, pers. obs.), which may predispose it relatively unresponsive to AM. In agreement with the negative effect on germination in the present experiment, a cocktail of the same AM fungi also reduced *Campanula rotundifolia* growth later on in the plant life (Nuortila et al. 2004).

In the present study, *Sibbaldia procumbens* adult plants reduced intraspecific seed germination and also seed germination in *Solidago* and *Campanula*. When monitoring the seedlings for 37-53 days, the presence of adult *Sibbaldia* reduced the growth rate of the *Campanula* seedlings, but not of the other three plant species (Kytöviita et al. 2003). This could be due to the adult *Sibbaldia* plants releasing secondary chemicals or reducing the soil nutrient levels. Moreover, the three AM fungi used did not modify these negative effects observed on the three plant species. Evidence for allelopathic effects mediated by AM fungi is recently building up (see e.g. Cipollini et al. 2012) even though the results are so far inconclusive. Conspecific allelopathy may be beneficial to plants as it may reduce competition due to avoiding generation overlap and reducing sibling rivalry. From the fungal point of view, because AM fungi are highly co-evolved obligate mutualists depending on host



plants, AM fungi should theoretically enhance seed germination regardless of the plant species, and therefore, we expected to see a positive effect of AM on seed germination for all plant species. The limited evidence obtained from the present study does not indicate a major role of AM fungi in mediating neighboring plant effects during seed germination. Experiments specifically designed to examine allelopathic effects and AM fungi should be carried out to fully comprehend the potential role of AM fungi in this regard.

Through affecting seed germination, AM fungi may act as a strong selective agent on the plant community composition. Our study shows that some plant-AM fungus combinations result in reduced seed germination while none enhanced germination. AM fungal symbiosis is generally seen as a mutualistic relationship and one must ask why would natural selection favor negative effects of AM on seed germination. One possible and testable hypothesis is that the presence of AM fungi does not reduce overall germination, but prolongs seed dormancy, which would be beneficial for both partners. Prolonged dormancy (i.e. slower germination rate) would be conceived as reduced germination rate in experiments like ours where the fate of the seeds that did not germinate was not investigated. Prolonged seed dormancy may be beneficial especially in habitats where seedling survival varies in time and bet-hedging results in highest recruitment into next generation such as desert, alpine or Arctic plant communities (see e.g. Philippi 1993, Venable 2007).

## Conclusions

We show that CMN may provide germinating seedlings faster acquisition of the AM partner in comparison to acquisition from spores. CMN membership may be beneficial to seedlings depending on specific environmental conditions and CMN properties. In contrast to host stimulated AM spore germination, an emerging pattern appears that AM fungi do not affect seed germination positively. AM fungi affect plants throughout all stages of the life history

and examining the effects at early life stage are critical to understand how vegetation composition is determined.

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**Table 1.** ANOVA results after fitting quasibinomial generalized linear models to the data on the proportion of seeds that germinated separately for each plant species.

	<i>Antennaria</i>			<i>Solidago</i>			<i>Sibbaldia</i>			<i>Campanula</i>		
<b>Factor</b>	F	dfs	<i>P</i>	F	dfs	<i>P</i>	F	dfs	<i>P</i>	F	dfs	<i>P</i>
Sibb	0.120	1,125	0.730	7.520	1,113	<b>0.007</b>	12.596	1,114	<b>&lt; 0.001</b>	13.024	1,118	<b>&lt; 0.001</b>
Fungi	1.059	3,122	0.369	1.436	3,110	0.236	1.004	3,111	0.394	6.090	3,115	<b>&lt; 0.001</b>
Sibb × Fungi	0.353	3,119	0.787	0.457	3,107	0.713	0.870	3,108	0.459	2.959	3,112	<b>0.035</b>

Sibb: Established *Sibbaldia*; Fungi: Fungal treatment.

**Table 2.** ANOVA results after fitting quasibinomial generalized linear models to the data on the proportion of seedlings showing AM fungal colonization separately for each plant species.

	<i>Antennaria</i>			<i>Solidago</i>			<i>Sibbaldia</i>			<i>Campanula</i>		
<b>Factor</b>	F	dfs	<i>P</i>	F	dfs	<i>P</i>	F	dfs	<i>P</i>	F	dfs	<i>P</i>
Age	39.916	1,140	<b>&lt;0.01</b>	-	-	-	28.380	1,134	<b>&lt;0.01</b>	1.252	1,129	0.27
Sibb	6.060	1,139	<b>0.02</b>	17.840	1,79	<b>&lt;0.01</b>	13.126	1,133	<b>&lt;0.01</b>	15.444	1,128	<b>&lt;0.01</b>
Fungi	0.131	2,141	0.88	1.520	2,80	0.23	17.393	2,135	<b>&lt;0.01</b>	1.993	2,130	0.14
Age × Sibb	0.001	1,134	0.98	-	-	-	0.058	1,128	0.81	1.425	1,123	0.24
Fungi × Age	1.083	2,137	0.34	-	-	-	1.886	2,131	0.16	3.372	2,126	<b>0.04</b>
Sibb × Fungi	0.565	2,135	0.57	4.334	2,77	<b>0.02</b>	9.710	2,129	<b>&lt;0.01</b>	6.420	2,124	<b>0.01</b>
Age × Sibb × Fungi	0.182	2,132	0.83	-	-	-	1.348	2,126	0.26	3.060	2,121	<b>0.05</b>

Sibb: Established *Sibbaldia*; Fungi: Fungal treatment.

**Table 3.** Publications reporting the effect of AM fungi on seed germination (excluding studies using fungicide treatments and those employing myco-heterotrophic plants).

Plant species	Seed mass <sup>1</sup>	AM fungal species	Effect	Reference
<i>Antennaria dioica</i>	0.06 g	<i>Glomus boreale</i> -like	ns	This study
		<i>Claroideoglomus claroideum</i>	ns	This study
		<i>Glomus hoi</i>	ns	This study
<i>Campanula rotundifolia</i>	0.06 g	<i>Glomus boreale</i> -like	↓ <sup>3</sup>	This study
		<i>Claroideoglomus claroideum</i>	↓ <sup>3</sup>	This study
		<i>Glomus hoi</i>	↓, ↑ <sup>3</sup>	This study
<i>Capsicum annuum</i>	6 g	<i>Glomus intraradices</i>	↑	Rueda-Puente et al 2010
<i>Cucumis sativus</i>	16.3 g	<i>Rhizophagus irregularis</i>	↓	Barber et al. 2013
<i>Geranium sylvaticum</i>	5.9 g	Natural soil community	↓	Varga 2015
<i>Gnaphalium norvegicum</i>	0.09 g	<i>Claroideoglomus claroideum</i>	ns	Ruotsalainen and Kytöviita 2004
<i>Leucanthemum vulgare</i>	0.42 g	<i>Funneliformis mosseae</i> + <i>Rhizophagus intraradices</i>	ns, ↓ <sup>2</sup>	Sadat Noori et al. 2014
<i>Phragmites australis</i>	0.09 g	<i>Funneliformis mosseae</i>	↓	Wu et al. 2014
		<i>Rhizophagus irregularis</i>	ns	Wu et al. 2014
<i>Sibbaldia procumbens</i>	0.45 g	<i>Glomus boreale</i>	ns	This study
		<i>Claroideoglomus claroideum</i>	ns	This study
		<i>Glomus hoi</i>	ns	This study
<i>Solidago virgaurea</i>	0.52 g	<i>Glomus boreale</i>	ns	This study
		<i>Claroideoglomus claroideum</i>	ns	This study
		<i>Glomus hoi</i>	ns	This study

ns: AM fungi did not affect seed germination; ↑ AM fungi increased seed germination; ↓ AM fungi decreased seed germination.

<sup>1</sup>Seed mass as mean for 1000 seeds, information obtained from the Seed Information Database, Kew Royal Botanic Gardens and McKeen and Richards (1996) for *P. australis*; <sup>2</sup>Observed effects were dependent on oil contamination level; <sup>3</sup>Observed effects were dependent on the presence of a CMN.

## Figure captions.

**Fig. 1.** Proportion of seeds from A) *Antennaria dioica* (N = 127 pots), B) *Solidago virgaurea* (N = 120 pots), C) *Sibbaldia procumbens* (N = 116 pots) and D) *Campanula rotundifolia* (N = 115 pots) that germinated when sown without (white boxes) or with (grey boxes) an established *Sibbaldia procumbens* plant in non-mycorrhizal conditions (NM) or with *Claroideoglomus claroideum* (CLA), *Glomus hoi* (HOI) or *Glomus boreale*-like (BOR) spores. Means  $\pm$  SE are indicated. Note the different scale of the y-axis for *Sibbaldia* versus the other plant species. Significant differences from pairwise comparisons due to the established *Sibbaldia* plant for each fungal treatments interaction are indicated with asterisks (\*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$ ).

**Fig. 2.** Proportion of 2-days old (left panels) and 6-days old (right panels) seedlings colonized by AM fungi in A) *Antennaria dioica*, B) *Solidago virgaurea*, C) *Sibbaldia procumbens* and D) *Campanula rotundifolia* germinated with (white bars) or without (black bars) an established *Sibbaldia procumbens* plant with *Claroideoglomus claroideum* (CLA), *Glomus hoi* (HOI) or *Glomus boreale*-like (BOR) spores. Means  $\pm$  SE are indicated. N is indicated above each bar.

**Fig. 3.** Relationship between the ratio of seeds germinated with AM fungi vs. without AM fungi and seed mass (log-transformed) from the studies presented in Table 3. The different studies are indicated with different symbols. The grey area indicates no significant effect due to the presence of AM fungi. Values above the grey area indicates that the presence of AM fungi increased seed germination; values below the grey area indicates that the presence of AM fungi decreased seed germination.

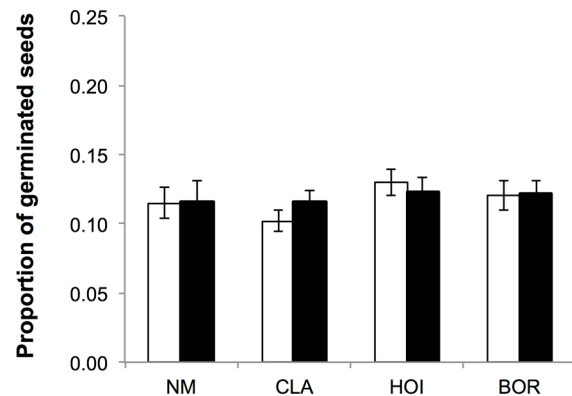
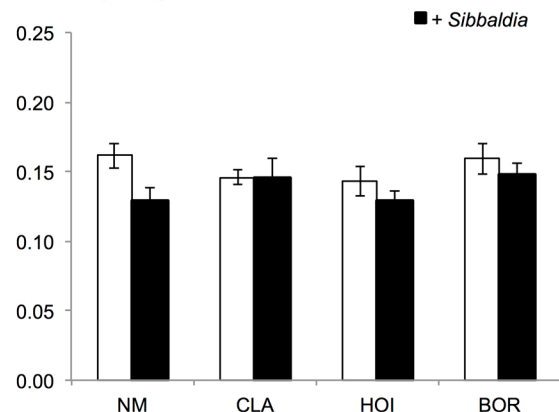
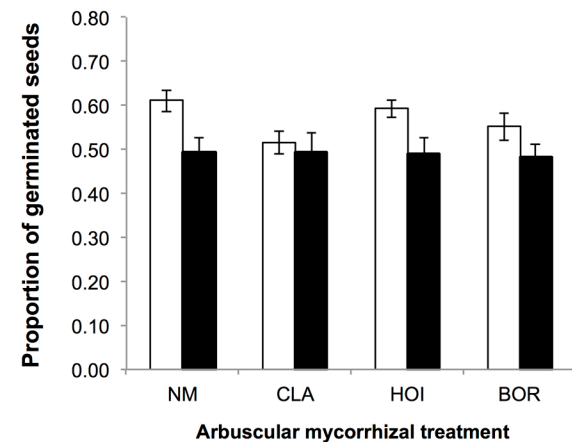
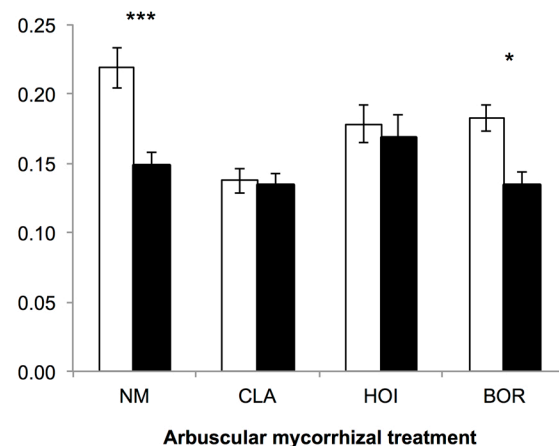
## Appendices.

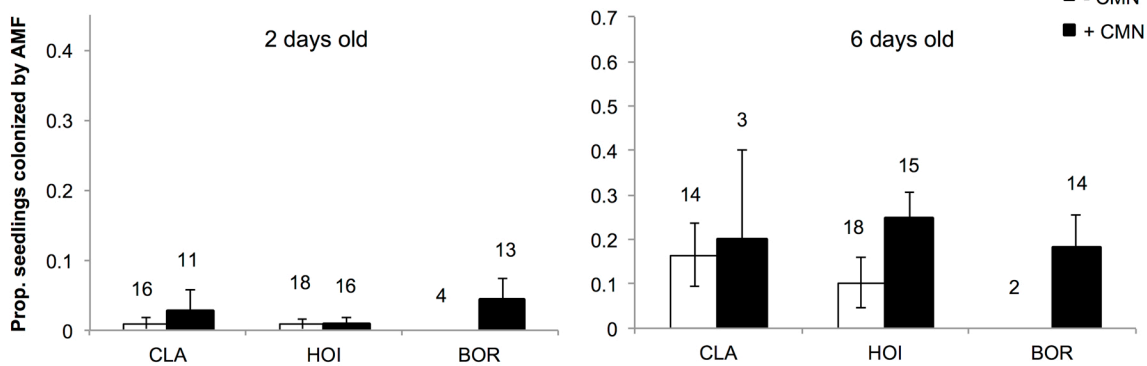
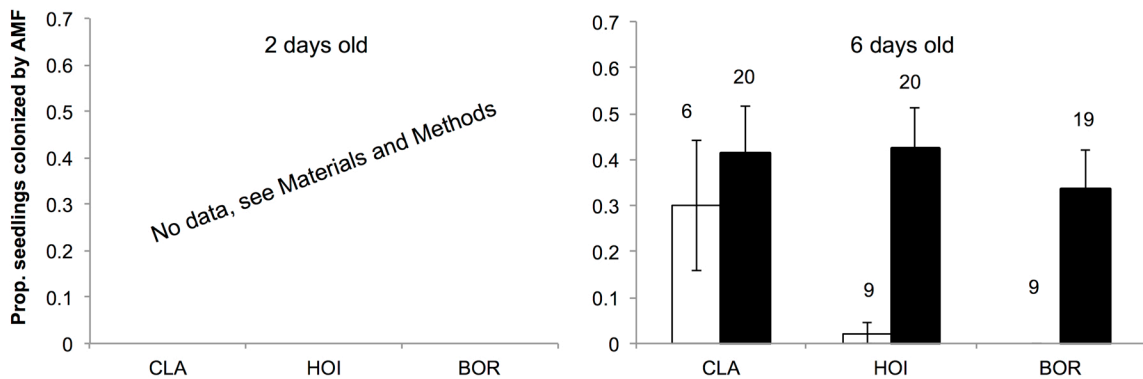
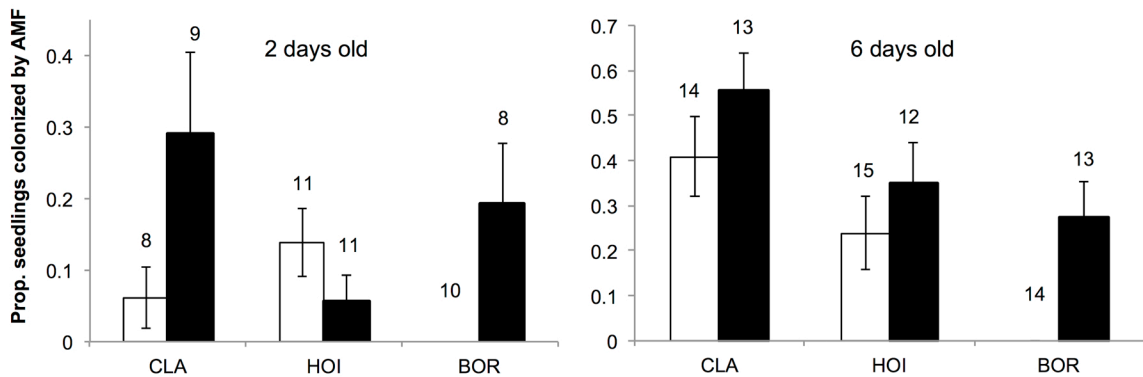
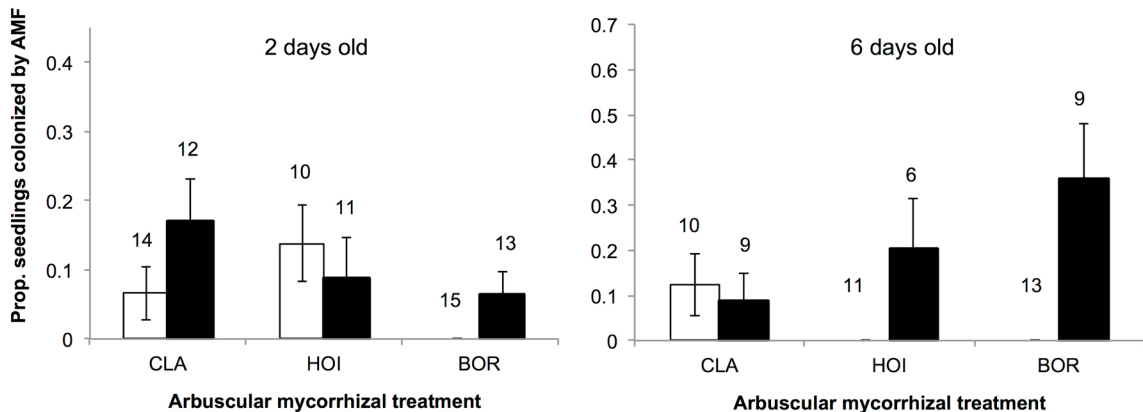
**Table A1.** ANOVA results after fitting a quasibinomial generalized linear model to the data on the proportion of seeds that germinated.

<b>Factor</b>	<b>F</b>	<b>dfs</b>	<b>P</b>
Plant species (Plants)	347.195	3,473	<b>&lt;0.001</b>
Established <i>Sibbaldia</i> (Sibb)	14.545	1,476	<b>&lt;0.001</b>
Fungal treatment (Fungi)	8.486	3,470	<b>&lt;0.001</b>
Sibb × Plants	4.417	3,467	<b>0.004</b>
Sibb × Fungi	3.663	3,464	<b>0.012</b>
Plants × Fungi	1.264	9,455	0.251
Sibb × Plants × Fungi	0.833	9,446	0.585

**Table A2.** ANOVA results after fitting a quasibinomial model to the data on the proportion of seedlings colonized by AM fungi.

<b>Factor</b>	<b>F</b>	<b>dfs</b>	<b>P</b>
Plant species (Plants)	12.733	3,493	<b>&lt;0.001</b>
Established <i>Sibbaldia</i> (Sibb)	62.496	1,496	<b>&lt;0.001</b>
Fungal treatment (Fungi)	9.505	2,498	<b>&lt;0.001</b>
Age	100.761	1,497	<b>&lt;0.001</b>
Sibb × Plants	0.919	3,476	0.432
Sibb × Fungi	19.999	2,489	<b>&lt;0.001</b>
Sibb × Age	0.889	1,488	0.346
Plants × Fungi	0.998	6,482	0.426
Plants × Age	5.351	3,479	<b>0.001</b>
Fungi × Age	0.590	2,491	0.555
Sibb × Plants × Fungi	1.189	6,464	0.311
Sibb × Age × Fungi	4.040	2,474	<b>0.018</b>
Age × Plants × Fungi	2.671	4,470	<b>0.030</b>
Sibb × Age × Plants	0.550	2,462	0.578
Sibb × Plants × Fungi × Age	0.340	4,458	0.851

**A. *Antennaria dioica*****B. *Solidago virgaurea*****C. *Sibbaldia procumbens*****D. *Campanula rotundifolia***

**A. *Antennaria dioica*****B. *Solidago virgaurea*****C. *Sibbaldia procumbens*****D. *Campanula rotundifolia***



Germination ratio (AM/NM)

